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Multicenter Survey of Routine Determinations of Resistance of *Helicobacter pylori* to Antimicrobials over the Last 20 Years (1990 to 2009) in Belgium▽

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We analyzed the rates of antimicrobial resistance of *Helicobacter pylori* strains isolated from patients from 1990 to 2009 and identified risk factors associated with resistance. Gastric biopsy specimens were collected from several digestive disease centers in Brussels, Belgium. We routinely performed antimicrobial susceptibility testing for clarithromycin (CLR), metronidazole, amoxicillin, tetracycline, and ciprofloxacin. Evaluable susceptibility testing was obtained for 9,430 strains isolated from patients who were not previously treated for *Helicobacter pylori* infection (1,527 isolates from children and 7,903 from adults) and 1,371 strains from patients who were previously treated (162 isolates from children and 1,209 from adults). No resistance to amoxicillin was observed, and tetracycline resistance was very rare (<0.01%). Primary metronidazole resistance remained stable over the years, with significantly lower rates for isolates from children (23.4%) than for isolates from adults (30.6%). Ciprofloxacin resistance remained rare in children, while it increased significantly over the last years in adults. Primary clarithromycin resistance increased significantly, reaching peaks in 2000 for children (16.9%) and in 2003 for adults (23.7%). A subsequent decrease of resistance rates down to 10% in both groups corresponded to a parallel decrease in macrolide consumption during the same period. Multivariate logistic regression revealed that female gender, age of the patient of 40 to 64 years, ethnic background, the number of previously unsuccessful eradication attempts, and the different time periods studied were independent risk factors of resistance to clarithromycin, metronidazole, and ciprofloxacin. Our study highlights the need to update local epidemiological data. Thus, the empirical CLR-based triple therapy proposed by the Maastricht III consensus report remains currently applicable to our population.

Helicobacter pylori is an etiologic agent of gastritis, peptic ulcer disease, gastric mucosa-associated lymphoid tissue lymphoma, and gastric adenocarcinoma (10, 27, 28). The eradication of *H. pylori* in infected patients is the most important goal in the management of *H. pylori*-associated diseases. Various drug regimens are recommended worldwide to treat *H. pylori* infection (10, 27). According to the last Maastricht III consensus report (27), 7- to 14-day triple therapy using a proton pump inhibitor (PPI) plus clarithromycin (CLR) and amoxicillin (AMX) or metronidazole (MET) remains the recommended first-choice treatment, although a sequential treatment is considered an alternative first choice (16). However, these treatments may fail for several reasons, particularly the resistance of *H. pylori* to antimicrobials. Bismuth-containing quadruple therapy, if available, is also a possible alternative first-choice treatment. Other regimens that have been proposed as second-line and/or rescue treatment include tetracycline (TET) and fluoroquinolones, for which resistances have also become an emerging issue (3, 47). Previously reported meta-analyses demonstrated overall risks of treatment failure of 55% and 25 to 37% for CLR- and MET-resistant isolates, respectively, using the classical triple therapy (13, 48). *H. pylori* resistance to fluoroquinolones has been associated with a 66.7% decrease in eradication rates (36). Neither the use of CLR nor the use of MET as a first-line drug is recommended in areas where more than 15 to 20% or 40% of the strains, respectively, are known to be resistant unless susceptibility has been tested previously (27). These data highlight the importance of the surveillance of antibiotic resistance of *H. pylori* strains in order to guide local and national prescribing policies. Data regarding *H. pylori* resistance in Belgium needed to be updated. The aim of this study was to assess and monitor the rates of antimicrobial resistance in *H. pylori* isolates obtained from several digestive disease centers in the region of Brussels, Belgium, over the last 2 decades in order to evaluate the trend in currently used antimicrobials and identify risk factors associated with resistance. Furthermore, the impact of the global consumption of antimicrobial drugs on resistance was analyzed, mutations con-

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ferring CLR resistance (CLR^r) were assessed for a subgroup of strains, and the frequencies of these mutations were compared to their frequencies in other European countries.

MATERIALS AND METHODS

From January 1990 to December 2009, *H. pylori* culture and antimicrobial susceptibility testing were performed using gastric biopsy specimens of patients who underwent upper gastrointestinal (GI) endoscopy for different clinical indications at different gastroenterology departments in Brussels, Belgium. Samples from children were collected at the Queen Fabiola Children's University Hospital and at the Saint-Pierre University Hospital, while samples from adults were collected at the CHIREC/site de la Basilique, at the Brugmann University Hospital, and at the Saint-Pierre University Hospital, as previously described (33). Other invasive tests for *H. pylori* detection were also performed during upper GI endoscopy (histology and rapid urease tests). The following items were also registered: age, gender, country of birth (and mother's country of birth for children), previous *H. pylori* treatment, and localization of the biopsy samples (antrum and/or fundus). According to age, the patients were classified into two groups: children (aged 0 to 17 years) and adults (aged ≥18 years). Primary (before the first specific anti-*H. pylori* treatment) and secondary (after at least one treatment failure) resistances were assessed according to available information regarding previous *H. pylori* treatment.

Isolation of *Helicobacter pylori*. Upon arrival at the laboratory, the gastric biopsy specimens were either frozen at −70°C until required or immediately processed according to previously described methods (18). Briefly, each specimen was ground in sterile water during 15 s. The final suspension was inoculated by circular streaking with a bent pipette onto in-house selective agar plates. Plates were incubated for 3 to 7 days at 37°C under a humid, microaerophilic incubator (Forma Scientific, Ann Arbor, MI, followed by Binder [serial no. 08-51907], Germany, for the last 2 years of the study) with a final atmosphere of 5 to 6% O_2, 8 to 10% CO_2, and 80 to 85% N_2. The culture was extended to a duration of 10 days if there was a known positive urea test. Compared to urea breath test and histology, this in-house culture method yielded 98% sensitivity and 100% specificity (9), and more recently, it was found to be equally efficient as and, moreover, more selective than 2 commercially available media (32).

Antimicrobial susceptibility testing. Susceptibility to nitroimidazoles (chiefly metronidazole [MET]), macrolides (chiefly clarithromycin [CLR]), fluoroquinolones (ciprofloxacin [CIP]), amoxicillin (AMX), and tetracycline (TET) was assessed under routine conditions using disk diffusion methods (Neo-Sensitabs; Rosco, Taastrup, Denmark), breakpoint susceptibility testing, and MIC determinations by an agar dilution method as previously described (4).

CIP susceptibility has been systematically tested only since 2003. Breakpoint susceptibility testing was systematically performed from October 1995 to November 2007 for CLR and MET, mainly to provide early information to clinicians. The main method allowing the final susceptibility testing was the disk diffusion method used for the currently tested antibiotics, which varies along the study according to the guidelines of treatment. In the absence of formal recommendations, owing to the important gap between MICs for susceptible and resistant *H. pylori* strains and according to a previous study of our population (18), strains were classified as susceptible to the antibiotics if growth inhibition zones were >40 mm without any mutant colony in the susceptibility area. The use of this constant breakpoint (up to the end of 2009) based on our clinical practice has the advantage of rendering the results homogeneous according to the definition of resistance. All the data were routinely collected and input into an Excel database.

Molecular analysis. In order to study the mechanism of resistance of *H. pylori* strains to CLR, we used molecular characterization. For this purpose we selected 637 CLR-resistant strains and 70 CLR-susceptible strains that served as controls. The three most frequently found point mutations (A2142C, A2142G, and A2143G) involved in CLR resistance were detected by real-time PCR according to a method described previously by Oleastro et al. (37). Briefly, a fragment of the 23S rRNA gene was amplified by primers specific for *H. pylori*, and probes labeled with LC-Red (37) and fluorescein were used for the real-time follow-up of the amplification by using fluorescence resonance energy transfer technology. PCR was then followed by a melting-curve analysis of the amplicons showing different melting temperature (T_m) values according to the wild type or mutation present. This molecular analysis was performed in Bordeaux (Centre National de Référence des Campylobacters et Helicobacters, Université Victor Segalen Bordeaux 2, France). In addition, the determination of MICs of CLR, MET, AMX, and moxifloxacin was performed with those 707 selected strains by using Etest strips (AB Biodisk, Solna, Sweden), which contain a predefined and continuous concentration gradient of each antibiotic. *H. pylori* strains were considered CLR resistant when the MICs were ≥1 μg/ml (34). In the absence of standard breakpoints, isolates were arbitrarily classified as resistant for cutoff values of ≥8 μg/ml, ≥2 μg/ml, and ≥1 μg/ml for MET, AMX, and moxifloxacin, respectively, as suggested previously by several authors (3, 6, 19, 31, 43).

Ecological study of antimicrobial consumption. From 1997 through 2009, national data for antibiotic consumption were obtained from Pharmanet, a section of the National Institute of Health and Disability Insurance (INAMI).

Data are expressed as the number of packages per 1,000 inhabitants per day. Beyond the study area, it represents nonhospital use for all of Belgium. Macrolide consumption data before 1997 were obtained from a previous report (4).

Data analysis. Univariate and multivariate analyses were performed with Statistical Package for Social Sciences, version 18. Logistic regression was performed with the forward method. Antibiotic susceptibility was introduced as a dependent variable. The covariates were gender (male [reference] or female), age groups (0 to 17 years, 18 to 39 years [reference], 40 to 64 years, or ≥65 years of age), period (6 periods for 1990 to 2007 by a range of 3 years and the period of 2008 to 2009 [reference]), previous eradication therapy (none [reference], 1, or 2 or more), and ethnic origin (Northern Europe, North Africa, Southern Europe, Middle East [reference], Central and West Africa, Eastern Europe, and Asia). As there was a strong interaction of previous *H. pylori* eradication therapy effects and period effects, a multivariate analysis for the period effect on antibiotic susceptibility was used for measurements in strata classified according to the presence or absence of a previous eradication attempt. Estimated odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. A P value of <0.05 indicated significant differences.

RESULTS

During the study period, 10,825 *H. pylori* isolates were collected from the different participating centers (1,696 strains isolated from children and 9,129 isolated from adults). Of these strains, 9,430 were isolated from patients who were not previously treated for *H. pylori* infection (1,527 from children and 7,903 from adults), 1,371 strains were isolated from patients who were previously treated for *H. pylori* infection (162 from children and 1,209 from adults), and we have no information concerning previous eradication attempts for 24 strains.

Antimicrobial susceptibility data were obtained for 10,670/10,825 isolates (for 155 *H. pylori* strains, the isolate was cultured but a subculture was not possible for antimicrobial susceptibility testing). A total of 58.0% (6,190/10,670 isolates) of the *H. pylori* strains were susceptible to all tested antimicrobials, 35.4% (3,774/10,670) were resistant to one drug, and 6.6% (702/10,670) were resistant to multiple drugs.

Primary and secondary antimicrobial resistance rates as well as patient characteristics are shown in Tables 1 and 2, respectively.

No difference was found between children and adult patients regarding gender ($P > 0.05$). Significant differences were found in patients' ethnic origins: adult patients originated mostly from Northern Europe (39%), while children were mainly from immigrant families from Northern Africa (52.3%) ($P < 10^{-4}$) (Table 1). The same patient characteristics were observed for the group of previously treated patients (Table 2).

For the group of patients who were never previously treated for *H. pylori* infection, 1,077 out of 1,527 strains (70.5%) isolated from children were susceptible to all tested antibiotics, compared to 4,783 out of 7,903 (60.5%) strains from adults ($P < 10^{-4}$) (Table 1). Resistance to only one antibiotic was observed for 25.1% of isolates from children and was observed for 38.8% of isolates from adults. Resistance to 2 antibiotics was observed for 2.9% of isolates from children and was ob-

TABLE 1. Comparison of data between children and adults for primary antimicrobial susceptibility and patient characteristics (n = 9,430)

Parameter	Value for group		OR (95% CI)	P
	Children	Adults		
No. of *H. pylori* isolates	1,527	7,903		
No. of males/no. of females	730/797	3,933/3,970	0.93 (0.83–1.03)	0.162
Median age (range) (yr)	10.6 (0.2–17.9)	48.0 (18.0–98.9)		
No. (%) of patients of ethnic background				
Northern Europe	152 (10.0)	3,085 (39.0)	0.18 (0.15–0.21)	<0.0001
Northern Africa	798 (52.3)	1,443 (18.3)	4.90 (4.37–5.50)	<0.0001
Southern Europe	93 (6.1)	879 (11.1)	0.52 (0.42–0.65)	<0.0001
Middle East	102 (6.6)	396 (5.0)	1.36 (1.08–1.70)	0.008
Central and West Africa	105 (6.8)	394 (5.0)	1.41 (1.13–1.76)	0.003
Eastern Europe	39 (2.6)	212 (2.7)	0.95 (0.67–1.34)	0.775
Asia	2 (0.1)	27 (0.3)	0.38 (0.10–1.46)	0.214
Unknown	236 (15.5)	1,467 (18.6)	0.80 (0.69–0.93)	0.004
No. (%) of isolates with antimicrobial susceptibility				
Susceptible to all antimicrobials	1,077 (70.5)	4,783 (60.5)	1.56 (1.39–1.76)	<0.0001
Resistant to MET	267 (17.4)	2,064 (26.1)	0.60 (0.52–0.69)	<0.0001
Resistant to CLR	111 (7.3)	413 (5.2)	1.42 (1.15–1.77)	0.002
Resistant to CIP	6 (0.4)	116 (1.5)	0.27 (0.12–0.59)	0.001
Resistant to MET and CLR	36 (2.4)	205 (2.6)	0.91 (0.64–1.30)	0.592
Resistant to MET and CIP	3 (0.2)	106 (1.3)	0.15 (0.05–0.43)	<0.0001
Resistant to CLR and CIP	4 (0.3)	47 (0.6)	0.44 (0.16–1.17)	0.126
Resistant to MET and TET	0 (0.0)	1 (0.0)	Not determined	
Resistant to MET, CLR, and CIP	0 (0.0)	45 (0.6)	0.00 (0.00–0.44)	0.001
Resistant to CLR, CIP, and TET	0 (0.0)	1 (0.0)	Not determined	
Susceptibility test failure	23 (1.5)	122 (1.6)	0.98 (0.63–1.52)	0.913

served for 4.5% of isolates from adults (Table 1). This significant difference in susceptibility between isolates from adults and those from children was also observed with higher resistance in the group of patients who had been previously treated, since in this group, 33.3% of isolates from children and 22% of isolates from adults were susceptible to all tested antimicrobials (Table 2).

Regarding specific molecules, there was no difference in the

TABLE 2. Comparison of data between children and adults for secondary antimicrobial susceptibility and patient characteristics (n = 1,371)

Parameter	Value for group		OR (95% CI)	P
	Children	Adults		
No. of *H. pylori* isolates	162	1,209		
No. of males/no. of females	77/85	592/617	0.94 (0.68–1.31)	0.732
Median age (range) (yr)	12.2 (2.3–17.8)	61.3 (18.0–90.2)		
No. (%) of patients of ethnic background				
Northern Europe	24 (14.8)	499 (41.3)	0.25 (1.16–0.39)	<0.0001
Northern Africa	81 (50.0)	232 (19.2)	4.21 (3.00–5.91)	<0.0001
Southern Europe	13 (8.0)	144 (11.9)	0.65 (0.36–1.16)	0.145
Middle East	24 (14.8)	55 (4.5)	3.65 (2.20–6.06)	<0.0001
Central and West Africa	6 (3.7)	37 (3.1)	1.22 (0.52–2.86)	0.659
Eastern Europe	4 (2.5)	37 (3.1)	0.80 (0.29–2.19)	0.678
Asia	0 (0.0)	4 (0.3)	Not determined	
Unknown	10 (6.2)	201 (16.6)	0.33 (0.17–0.63)	0.001
No. (%) of isolates with antimicrobial susceptibility				
Susceptible to all antimicrobials	54 (33.3)	266 (22.0)	1.77 (1.25–2.52)	0.002
Resistant to MET	61 (37.7)	593 (49.0)	0.63 (0.45–0.88)	0.006
Resistant to CLR	23 (14.2)	102 (8.5)	1.80 (1.11–2.91)	0.017
Resistant to CIP	1 (0.6)	9 (0.7)	0.83 (0.14–5.10)	1
Resistant to MET and CLR	20 (12.4)	142 (11.7)	1.06 (0.65–1.74)	0.824
Resistant to MET and CIP	2 (1.2)	22 (1.8)	0.67 (0.17–2.61)	0.594
Resistant to CLR and CIP	0 (0.0)	25 (2.1)	0.00 (0.00–1.13)	0.063
Resistant to MET and TET	0 (0.0)	0 (0.0)	Not determined	
Resistant to MET, CLR, and CIP	0 (0.0)	43 (3.6)	0.00 (0.00–0.64)	0.007
Resistant to CLR, CIP, and TET	0 (0.0)	0 (0.0)	Not determined	
Susceptibility test failure	1 (0.6)	7 (0.6)	1.07 (0.17–6.71)	1

TABLE 3. Factors associated with clarithromycin resistance (n = 10,670)

Parameter	Value		OR (95% CI)	Adjusted OR (95% CI)	Adjusted P
	Resistant	Susceptible			
No. of *H. pylori* isolates	1,220	9,450			
No. of males/no. of females (males = reference)	534/686	4,734/4,716	1.29 (1.14–1.45)	1.21 (1.07–1.38)	0.003
No. (%) of patients in age group (yr)					0.001
0–17	194 (11.6)	1,477	1.19 (0.99–1.45)	0.92 (0.75–1.13)	0.411
18–39	295 (9.9)	2,679	1 (ref)	1 (ref)	
40–64	538 (13.2)	3,525	1.39 (1.19–1.61)	1.29 (1.10–1.51)	0.002
65–98	193 (9.8)	1,769	0.99 (0.82–1.20)	1.02 (0.83–1.27)	0.818
No. (%) of patients of ethnic background					0.041
Northern Europe	405 (10.9)	3,298	1.13 (0.84–1.52)	1.41 (1.03–1.94)	0.033
Northern Africa	290 (11.5)	2,239	1.19 (0.88–1.61)	1.14 (0.83–1.57)	0.415
Southern Europe	143 (12.8)	972	1.36 (0.98–1.88)	1.39 (0.98–1.96)	0.062
Middle East	56 (9.8)	516	1 (ref)	1 (ref)	
Central and West Africa	64 (11.8)	478	1.23 (0.85–1.80)	0.99 (0.66–1.47)	0.947
Eastern Europe	33 (11.5)	254	1.20 (0.76–1.88)	0.99 (0.62–1.60)	0.975
Asia	5 (15.6)	27	1.71 (0.65–4.47)	2.61 (0.89–7.61)	0.079
Unknown	224 (11.9)	1,666	1.24 (0.91–1.69)	1.34 (0.96–1.85)	0.082
No. (%) of patients with previous eradication attempt					0.000
None	862 (9.3)	8,423	1 (ref[a])		
1	309 (25.4)	909	3.32 (2.87–3.85)	4.74 (4.04–5.58)	0.000
2 or more	46 (31.7)	99	4.54 (3.18–6.48)	6.82 (4.6–10.08)	0.000
No. (%) of isolates during time period					
Before eradication attempt					
1990–1992	35 (2.0)	1,752	0.17 (0.12–0.26)	0.15 (0.10–0.23)	0.000
1993–1995	83 (5.2)	1,523	0.47 (0.34–0.64)	0.43 (0.31–0.59)	0.000
1996–1998	177 (10.3)	1,539	0.99 (0.75–1.29)	0.92 (0.70–1.21)	0.563
1999–2001	166 (13.5)	1,063	1.34 (1.02–1.76)	1.27 (0.96–1.68)	0.090
2002–2004	150 (15.3)	832	1.55 (1.17–2.05)	1.51 (1.14–2.01)	0.004
2005–2007	163 (14.5)	960	1.46 (1.11–1.92)	1.45 (1.10–1.97)	0.008
2008–2009	88 (10.5)	754	1 (ref)		
After eradication failure					
1990–1992	24 (5.6)	402	0.05 (0.03–0.08)	0.05 (0.03–0.08)	0.000
1993–1995	48 (15.5)	262	0.15 (0.10–0.24)	0.15 (0.09–0.23)	0.000
1996–1998	65 (37.1)	110	0.49 (0.32–0.76)	0.48 (0.31–0.75)	0.001
1999–2001	62 (47.3)	69	0.74 (0.47–1.18)	0.79 (0.49–1.26)	0.323
2002–2004	28 (37.3)	47	0.49 (0.28–0.86)	0.54 (0.308–0.96)	0.037
2005–2007	41 (47.1)	46	0.74 (0.44–1.24)	0.89 (0.52–1.54)	0.688
2008–2009	87 (54.7)	72	1 (ref)		

[a] ref, reference odds ratio value of 1 arbitrarily attributed to the group to which the others were compared.

rates of resistance to CLR between strains from children and those from adults. On the contrary, resistance rates were highly significantly different for MET (23.4% in strains from children compared to 35.9% in strains from adults [$P < 10^{-4}$]) (see Table 4) and CIP (2.0% in strains from children compared to 17.5% in strains from adults [$P < 10^{-4}$]) (see Table 5).

Risk factors for resistance. (i) Amoxicillin. Of all the isolates routinely tested using the disk diffusion method, none were found to be resistant to amoxicillin. The MICs remained stable over the 2 decades, with an MIC_{90} of 0.094 μg/ml. However, out of the 707 isolates selected for retrospective molecular analysis and MIC determinations, 4 (0.6%) exhibited an increased AMX MIC with intermediate resistance (2 μg/ml > MIC > 0.5 μg/ml).

(ii) Tetracycline. Only two isolates (<0.01%) were resistant to tetracycline and only in combination with other antimicrobial resistances (Table 1). The first one was isolated in 1997 from a 19-year-old sub-Saharan African (male) patient. The second one was isolated 10 years later, from a 63-year-old Belgian (male) patient. Both patients had not received any previous treatment for *H. pylori* eradication.

(iii) Clarithromycin. Data for the analysis of risk factors associated with CLR resistance (CLR^r) are shown in Table 3, which included data from both the univariate analysis and multivariate analysis by logistic regression. All the covariates (period, ethnicity, gender, age, and previous eradication therapy) had a significant effect on CLR^r. The overall rate of CLR^r was higher for females, with resistance rates of 12.7%, compared to 10.1% among males (P [adjusted] = 0.003). The difference was higher for the group of untreated patients, especially among adult patients: 10.4% (408/3,501 isolates) for females and 7.8% (303/3,569) for males ($P < 10^{-4}$; OR, 1.37 [95% CI, 1.17 to 1.60]).

The CLR^r rate was significantly higher for the group includ-

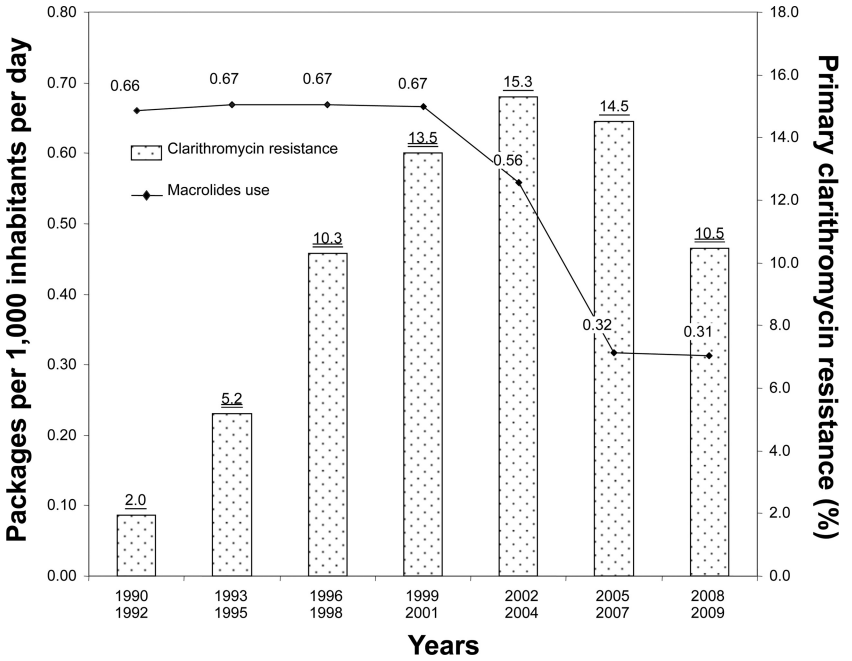

FIG. 1. Outpatient macrolide use (expressed as number of packages per 1,000 inhabitants per day) and evolution of primary *Helicobacter pylori* resistance to clarithromycin over the years of the study.

ing patients 40 to 64 years old (13.9%) than for young adults (9.9%).

The multivariate analysis showed a significant difference in CLR^r rates between Northern European (1.4-fold risk increase) and Middle Eastern patients.

A significant difference in CLR^r between treated and untreated patients was found. Moreover, compared to that of the group without any previous treatment, the risk of CLR^r was 4.7 times higher after one eradication failure and 6.8 times higher after at least two previous eradication attempts.

The rate of secondary CLR^r increased gradually and significantly, from 5.6% at the beginning of the study to 54.7% during the last 2 years.

Although primary CLR^r was rare at the beginning of the study, the rate of primary CLR^r increased significantly up to the year 2003 (19.2%) and then decreased substantially, reaching 9.9% in 2009. The peak values of the CLR^r rate were observed in 2000 for children (16.9%) and in 2003 for adults (23.7%). This resistance trend in the population correlated with outpatient macrolide consumption, expressed as the number of packages per 1,000 inhabitants per day, which decreased by 50% between 2001 and 2009 (Fig. 1).

CLR-susceptible isolates generally exhibited low MICs of <0.016 μg/ml. A range of MICs was observed for the resistant phenotypes, with high-level resistance (>256 μg/ml) accounting for 68.1% (434 out of 637) of resistant isolates. No significant difference in MIC distributions was observed between the child and the adult groups (data not shown).

The characterization of CLA^r using real-time PCR revealed that 20 strains (3.1%) of the 637 CLR-resistant strains (MICs ≥ 1 μg/ml) had the A2142C mutation, while the rest exhibited either the A2142G or the A2143G mutation. The distributions of the mutations involved were the same for children and adults. Of the 70 CLR-susceptible strains (MICs < 1 μg/ml) tested for control purposes, a mutation was detected in 3 cases (A2142/43G). These discrepancies were clarified later by additional subcultures and new PCR analyses, revealing that 123 isolates (19.2%) yielded both wild-type and resistant profiles.

The 20 strains yielding the A2142C mutation had very high CLR MICs (>256 μg/ml). On the contrary, no correlation between MICs and A2142G/43G mutations could be found.

(iv) Metronidazole. Table 4 shows the effects of time period of isolation, ethnicity, gender, age, and previous eradication on *H. pylori* resistance to MET (MET^r). Prior to eradication, the level of MET^r remained stable over the 2 decades, with average rates of resistance of 30.6% and 23.4% for adults and children, respectively ($P < 10^{-4}$). A failure of treatment increased the risk of harboring MET^r isolates 4.3-fold for one treatment failure and 7.3-fold for two or more failures.

The time period of isolation was a significant risk factor in the multivariate analysis by the logistic regression model for the group of previously treated patients: the risk of secondary infection by a MET^r isolate was 3.7-fold higher at the beginning of the study; however, a significant decrease of the resistance rate was observed during the following years, particularly for children (89.5% for the first years to 39.1% for the last years [$P < 10^{-4}$]). Female gender was a significant independent risk factor for MET^r, showing 1.5-fold more risk by logistic regression. On the other hand, the Asian and West and Central African ethnic groups were the two ethnic groups with a higher risk of MET^r (ORs, 2.9 and 2.6, respectively, in the multivariate model). Finally, MET^r was higher for the strains isolated from patients aged 40 to 64 years.

Six out of 707 isolates (0.8%) showed discrepant susceptibility results between the disk diffusion method and MIC de-

TABLE 4. Factors associated with metronidazole resistance (n = 10,648)

Parameter	Value		OR (95% CI)	Adjusted OR (95% CI)	Adjusted P
	Resistant	Susceptible			
No. of *H. pylori* isolates	3,610	7,038			
No. of males/no. of females (males = reference)	1,566/2,044	3,688/3,350	1.44 (1.32–1.60)	1.52 (1.40–1.66)	0.000
No. (%) of patients in age group (yr)					0.000
0–17	389 (23.4)	1,276	0.51 (0.45–0.59)	0.52 (0.45–0.61)	0.000
18–39	1,103 (37.2)	1,863	1		
40–64	1,606 (39.6)	2,449	1.11 (1.01–1.22)	1.06 (0.96–1.18)	0.263
65–98	512 (26.1)	1,450	0.60 (0.53–0.68)	0.59 (0.51–0.68)	0.000
No. (%) of patients of ethnic background					0.000
Northern Europe	1,179 (31.9)	2,520	0.93 (0.77–1.12)	0.90 (0.74–1.10)	0.309
Northern Africa	706 (28.0)	1,814	0.78 (0.64–0.94)	0.83 (0.67–1.01)	0.089
Southern Europe	431 (38.7)	682	1.26 (1.02–1.56)	1.21 (0.96–1.51)	0.099
Middle East	191 (33.4)	381	1		
Central and West Africa	305 (56.8)	232	2.62 (2.05–3.35)	2.91 (2.26–3.76)	0.000
Eastern Europe	104 (36.4)	182	1.14 (0.85–1.53)	1.11 (0.81–1.51)	0.522
Asia	19 (59.4)	13	2.91 (1.4–6.03)	2.84 (1.35–5.98)	0.006
Unknown	675 (35.7)	1,214	1.11 (0.91–1.35)	1.12 (0.91–1.38)	0.269
No. (%) of patients with previous eradication attempt					
None	2,727 (29.4)	6,558	1		
1	776 (63.7)	442	4.22 (3.73–4.78)	4.38 (3.85–4.98)	0.000
2 or more	107 (73.8)	38	6.77 (4.66–9.83)	7.30 (4.99–10.68)	0.000
No. (%) of isolates during time period					
Before eradication attempt					0.012
1990–1992	504 (28.2)	1,283	1.01 (0.84–1.21)	1.12 (0.92–1.36)	0.242
1993–1995	452 (28.1)	1,154	1.01 (0.83–1.21)	1.12 (0.92–1.36)	0.270
1996–1998	471 (27.4)	1,245	0.97 (0.81–1.17)	1.06 (0.9–1.28)	0.582
1999–2001	397 (32.3)	832	1.22 (1.01–1.48)	1.34 (1.10–1.64)	0.004
2002–2004	309 (31.5)	673	1.18 (0.96–1.44)	1.33 (1.08–1.64)	0.007
2005–2007	358 (31.9)	765	1.20 (0.99–1.46)	1.18 (0.96–1.44)	0.119
2008–2009	236 (28.0)	606	1		
After eradication failure					0.000
1990–1992	354 (83.1)	72	3.31 (2.21–4.97)	3.71 (2.43–5.66)	0.000
1993–1995	186 (60.0)	124	1.01 (0.68–1.49)	1.13 (0.75–1.70)	0.557
1996–1998	93 (53.1)	82	0.76 (0.49–1.18)	0.83 (0.53–1.30)	0.428
1999–2001	81 (61.8)	50	1.09 (0.68–1.75)	1.15 (0.71–1.88)	0.567
2002–2004	39 (52.0)	36	0.73 (0.42–1.27)	0.75 (0.43–1.33)	0.333
2005–2007	35 (40.2)	52	0.45 (0.27–0.77)	0.51 (0.30–0.89)	0.018
2008–2009	95 (59.7)	64	1		

terminations using the Etest. MICs were stable over the entire study period, with an MIC_{50} of 0.5 μg/ml.

(v) Ciprofloxacin. The effects of covariables on *H. pylori* resistance to CIP (CIP^r) are shown in Table 5. As for CLR and MET, female gender, age of the patient of 40 to 64 years, previous treatment, and number of previous eradication attempts were associated with a higher risk of infection by a CIP^r isolate.

CIP^r increased gradually and significantly ($P < 10^{-4}$) over the study period: from 13% and 18.8% in 2003 to 15.6% and 38.6% in 2009 for primary and secondary resistances, respectively. The moxifloxacin MIC determinations performed retrospectively for selected strains did not show any resistant strains during the first years of the study. Fluoroquinolone resistance remained rare for isolates from children. The first two cases of resistance were observed respectively in 2004 and 2009 for an untreated child and a treated child. *H. pylori* strains from Central and West African patients exhibited the highest levels of CIP^r.

No correlation was found between CIP resistance rates and outpatient use of fluoroquinolones nationally.

Multiple resistances. No strain was resistant to more than three out of the five antibiotics tested in this study.

All the strains exhibiting triple resistance (CLR, MET, and CIP) were strains from adults (Table 1).

H. pylori strains were resistant to multiple drugs in 4.7% and 18.5% of cases, before (Table 1) and after (Table 2) treatment, respectively ($P < 10^{-4}$).

Multiple-drug resistances increased significantly over the study period. Indeed, in 2009 we observed 22.2% and 39.8% overall dual CLR-MET, 13.6% and 20.5% dual CLR-CIP, and 6.3% and 13% triple CLR-MET-CIP resistance rates in 2003 and 2009, respectively ($P < 10^{-4}$).

DISCUSSION

Resistance to antimicrobials jeopardizes *H. pylori* eradication (10, 27). Our 20-year historical descriptive study was performed to assess the interrelationship between antibiotic resistance and age, gender, period of observation, ethnic/

TABLE 5. Factors associated with ciprofloxacin resistance (n = 2,933)

Parameter	Value		OR (95% CI)	Adjusted OR (95% CI)	Adjusted P
	Resistant	Susceptible			
No. of isolates	389	2,544			
No. of males/no. of females (male = reference)	155/234	1,153/1,391	1.25 (1.01–1.55)	Out of criteria	
No. (%) of patients in age group (yr)					0.000
0–17	16 (2.0)	768	0.15 (0.09–0.26)	0.14 (0.08–0.25)	0.000
18–39	107 (12.3)	763	1		
40–64	202 (20.6)	779	1.85 (1.43–2.39)	1.75 (1.34–2.29)	0.000
65–98	64 (21.5)	234	1.95 (1.38–2.75)	2.26 (1.55–3.28)	0.000
No. (%) of patients of ethnic background					0.023
Northern Europe	93 (17.8)	430	1.04 (0.63–1.7)	0.62 (0.36–1.07)	0.085
Northern Africa	94 (9.2)	924	0.49 (0.3–0.8)	0.59 (0.35–0.99)	0.047
Southern Europe	49 (15.0)	278	0.84 (0.49–1.43)	0.60 (0.34–1.07)	0.085
Middle East	24 (17.3)	115	1		
Central and West Africa	59 (19.5)	244	1.16 (0.69–1.96)	1.01 (0.57–1.77)	0.978
Eastern Europe	12 (9.6)	113	0.51 (0.24–1.07)	0.41 (0.19–0.90)	0.027
Asia	0	5	Not determined		
Unknown	58 (11.8)	435	0.64 (0.38–1.07)	0.53 (0.30–0.92)	0.025
No. (%) of patients with previous eradication attempt					0.000
None	295 (11.2)	2,340	1		
1	79 (29.9)	185	2.39 (1.79–3.19)	3.30 (2.40–4.53)	0.000
2 or more	15 (44.1)	19	6.26 (3.15–12.45)	5.62 (2.60–12.14)	0.000
No. (%) of isolates during time period					
Before eradication attempt					0.020
2002–2004	58 (8.6)	613	0.67 (0.48–0.94)	0.61 (0.43–0.86)	0.006
2005–2007	133 (11.8)	990	0.95 (0.72–1.25)	0.88 (0.86–1.17)	0.369
2008–2009	104 (12.4)	737	1		
After eradication failure					0.041
2002–2004	8 (15.4)	44	0.26 (0.11–0.59)	0.32 (0.13–0.79)	0.014
2005–2007	21 (24.1)	66	0.46 (0.26–0.82)	0.87 (0.33–1.36)	0.267
2008–2009	65 (40.9)	94	1		

geographic origin, and previous treatment of *H. pylori* infection.

As found for many other countries, *H. pylori* resistance to TET and AMX was very infrequent in our population (6, 14, 31, 44, 50). Therefore, our analysis focused on resistance to CLR, MET, and CIP.

Effect of age on antibiotic resistance. Throughout the study, the resistance rates were always lower for children than for adults. This was the most obvious for fluoroquinolones, since these molecules are rarely used to treat children. A Bulgarian survey also found lower rates of MET^r in isolates from children than in isolates from adults (6). Concerning CLR, our findings contrast with the results reported previously for other studies that disclosed a higher CLR resistance rate for strains isolated from children (1, 2, 6, 8, 19, 46). Even after the further stratification of children into subgroups (<6 years, 6 to 12 years, and 13 to 17 years of age), resistance rates were lower for children below the age of 6 years, but the difference did not reach any significance through these subgroups. Concerning the Etest performed with selected strains, we found a trend for higher CLR MICs only for isolates from children, but the differences were not significant (data not shown).

Effect of period of observation on antibiotic resistance. Throughout the study period, we observed the evolution of CLR^r before treatment for 1,220 children and 9,450 adults. Compared to those of other European countries, the mean primary resistance rate observed for Brussels (11%) was low (43, 53). Although very rare at the beginning of the study, rates of primary CLR^r increased significantly and reached maximum rates in 2000 for children (16.9%) and in 2003 for adults (23.7%). Part of the data pointing out this dramatic increase of CLR^r among isolates from children between 1989 and 2000 was reported previously (4). The gradual increase in levels of resistance is probably also related to the widespread use of macrolides to treat other diseases, particularly respiratory tract infections in Belgium, and the introduction of new macrolides around 1995, such as CLR and azithromycin (7). In contrast with our findings, a decrease in rates of CLR^r was observed in the Netherlands between 1997 and 2002 (22), rates of CLR^r did not change over the period of 1994 to 2005 for French children (23), and they did not decrease between 1996 and 2007 for Bulgarian children (5, 6).

The trend of the decline in rates of primary CLR^r observed during the last years of the study (particularly in 2008 and 2009, with a mean value of 10.5% resistant strains) contrasts with the constant increases observed for other European countries, such as Austria, Bulgaria, France, and Italy (6, 12, 15). The observed decrease in rates of primary resistance may be mul-

tifactorial. However, it is certainly related mainly to the observed decrease in the rate of macrolide consumption, as shown in Fig. 1. Between 2000 and 2003 the Belgian Antibiotic Policy Coordination Committee (BAPCOC) launched two campaigns for the rational use of antibiotics in Belgium, followed by the national restriction of the usage of broad-spectrum antimicrobials (20). This explanation is reinforced by the fact that a decrease in rates of macrolide resistance was also found for *Streptococcus pyogenes* and *Streptococcus pneumoniae* by the BAPCOC (20). It is well known that the use of long-acting macrolides such as azithromycin induces a resistance rate higher than those induced by other macrolides. Individual data concerning the consumption of macrolides in Belgium have been available from the records of the National Institute of Health since 1997. Although the consumption rates showed variability and, particularly, an important decrease since 2000, azithromycin and clarithromycin each represent one-third of the total macrolide consumption. The proportion between these 2 molecules has remained stable throughout the 12-year observation period. The mean primary rates of resistance to MET remained stable over the study period for the whole group of untreated patients, as found previously for other European countries (22, 50). Our data point out a gradual and significant decrease in rates of secondary MET^r (83.1% to 59.7%). MET is the oldest molecule used for *H. pylori* eradication in Belgium. This could explain the highest rates of secondary resistance observed at the beginning of the study (93% and 83% for treated children and adults, respectively). The introduction of triple therapy with a PPI, AMX, and CLR in the treatment regimens has probably reduced the use of MET for *H. pylori* eradication in our population and could explain the lower rate of resistance observed thereafter for treated patients. The limitations of existing therapies related to the increasing resistance of *H. pylori* strains to MET and CLR worldwide have led to the proposition of alternative regimens involving agents such as rifamycin derivatives and fluoroquinolones (17, 27). The latter were introduced in Belgian practices in 2005 for *H. pylori* "rescue therapy." Consequently, we gradually found a significant increase in rates of CIP^r *H. pylori* infection in adults. A similar trend was recently reported in Europe (6). This increase is probably related to the increasing use of fluoroquinolones in the community. Regarding the higher resistance rates observed for the last years of the study, particularly for previously treated patients, the impact of the use of fluoroquinolones on *H. pylori* eradication is disappointing. As a consequence, the multiresistant *H. pylori* strains have currently become a very serious problem (20% after treatment failure). High rates of fluoroquinolone resistance have also been reported by other centers in Belgium (3), France (43), and Italy (53). In the current survey, the first cases of CIP^r in isolates from children occurred in 2004, when the resistance rate was particularly high for adult patients. This finding is probably related to intrafamilial transmission or other possibilities of transmitting strains from adults to children. This assessment should be confirmed by further molecular typing analyses.

Effect of previous antibiotic treatment of *H. pylori* infection. Previous treatment with CLR is the most important risk factor for the development of secondary resistance in *H. pylori* (10, 27). Indeed, in the group of treated patients, a gradual and significant increase in the CLR^r rate was observed (5.6% to 54.7%) (Table 3) over the 2 decades under study. It is probably partly related to the fact that CLR is a first-choice drug in the practices of the majority of Belgian gastroenterologists.

As described previously (1, 14, 26), a significant difference between treated and untreated patients was reported for CLR, MET, and CIP, probably related to dual and triple resistances. Moreover, over the study period, we observed a gradual increase in multiple-drug-resistant strains, probably reflecting multiple unsuccessful attempts at eradication.

These are the arguments stressing the need for susceptibility testing prior to the implementation of a wide policy for *H. pylori* eradication.

Some of the cases included in the group of secondary resistance may have been successfully treated in the past and then reinfected, since 23.3% of these cases were infected with *H. pylori* strains that were susceptible to all the tested antimicrobials (Table 2). However, in most cases (>80%), an assessment of the success of therapy was performed within 2 to 3 months, so they may be considered treatment failures. Therefore, eradication failure could be related to other factors, particularly the lack of an adherence of patients to treatment (21).

Effect of gender. We found that female gender seems to exert a significant influence on CLR^r, MET^r, and CIP^r. It is particularly obvious in adults, since the age group with the maximum risk of resistance is patients 40 to 64 years old. The influence of gender on *H. pylori* resistance is controversially documented. A previously reported multicenter study of children living in Europe showed a significant gender difference in CLR^r, with a higher level of resistance for males (26). Other studies did not show a significant gender difference (29, 53). Finally, lower rates of resistance to MET or/and CLR in male patients have been reported all over the world (25, 40, 42, 45, 50, 52). High rates of MET resistance in females could be due to the use of this drug to treat gynecological infections. It was described previously that mutations associated with CLR in *H. pylori* could confer cross-resistance to other agents in the macrolide, lincosamide, and streptogramin (MLS) antibiotic group (51). The significantly higher level of resistance to CLR observed for female adults could then be related to the treatment of gynecological infections, because clindamycin is also used for the treatment of bacterial vaginosis.

Effect of ethnicity. This is the first Belgian report, to our knowledge, comparing *H. pylori* resistance rates and ethnic backgrounds of the patients. We observed higher CLR^r rates for patients of European origin than for those of Middle Eastern origin, which is probably related to higher rates of macrolide consumption in Europe. In Africa, MET is often used to cure parasitological infections (amebiasis, giardiasis, and trichomoniasis), with dramatic consequences for *H. pylori* resistance. Indeed, the highest MET^r rates (>90%) have been reported for Central Africa (35, 39). This could also explain the higher MET^r rates observed for *H. pylori* strains isolated from patients coming from West and Central African countries. These ethnic differences were also described previously by a prospective multicenter study of children living in Europe (26). Consequently, according to the Maastricht III consensus report, MET should not be used prior to susceptibility testing of patients originating from Asia and sub-Saharan Africa.

Phenotypic and molecular susceptibility testing. Treatment with macrolides induces point mutations in the 23S rRNA gene that could result in the acquisition of resistance (41). Despite the heterogeneity of our population (33), mutations involved in CLR^r were comparable to mutations reported previously by other studies in Europe, which were mainly A2142G/43G mutations and various combinations of the wild type as well as point mutations related to the presence of multiple isolates (mixed population) in the same biopsy specimen (1, 12, 31, 37, 43, 44, 49). In contrast with data from many previous studies (1, 2, 11, 24), our study identified the A2142C mutation in some strains from children, probably due to the large number of isolates in our study. The A2142C mutation was associated with a higher level of resistance (CLR MIC of >256 μg/ml), with half of the strains involved being related to patients with at least 2 documented eradication failures. However, the clinical significance of this observation for the risk of treatment failure needs further confirmation and assessment in prospective studies.

As found in this study, high MICs are a common finding for CLR^r strains (26). This finding illustrates the fact that the impact of resistance on clinical outcome is dramatic (13, 30).

Concerning MET, we observed few discrepant results between the MIC determinations and the disk diffusion method in assessing MET^r. This is probably related to the fact that Etest results for MET overestimate the presence of resistance (31, 38).

In conclusion, this study highlights the need to update local epidemiological data for *H. pylori* antimicrobial resistance to optimize the efficacy of eradication strategies. According to the Maastricht III consensus report, empirical CLR-based triple therapy remains applicable to our population, since we found a significant decrease in primary resistance that seems correlated with the lesser use of macrolides.

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REFERENCES

1. **Agudo, S., G. Perez-Perez, T. Alarcon, and M. Lopez-Brea.** 2010. High prevalence of clarithromycin-resistant *Helicobacter pylori* strains and risk factors associated with resistance in Madrid, Spain. J. Clin. Microbiol. **48:** 3703–3707.
2. **Alarcon, T., A. E. Vega, D. Domingo, M. J. Martinez, and M. Lopez-Brea.** 2003. Clarithromycin resistance among *Helicobacter pylori* strains isolated from children: prevalence and study of mechanism of resistance by PCR-restriction fragment length polymorphism analysis. J. Clin. Microbiol. **41:** 486–499.
3. **Bogaerts, P., C. Berhin, H. Nizet, and Y. Glupczynski.** 2006. Prevalence and mechanisms of resistance to fluoroquinolones in *Helicobacter pylori* strains from patients living in Belgium. Helicobacter **11:**441–445.
4. **Bontems, P., et al.** 2001. Twelve year observation of primary and secondary antibiotic-resistant *Helicobacter pylori* strains in children. Pediatr. Infect. Dis. J. **20:**1033–1038.
5. **Boyanova, L., et al.** 2008. Prevalence and evolution of *Helicobacter pylori* resistance to 6 antibacterial agents over 12 years and correlation between susceptibility testing methods. Diagn. Microbiol. Infect. Dis. **60:**409–415.
6. **Boyanova, L., et al.** 2010. Two-decade trends in primary *Helicobacter pylori* resistance to antibiotics in Bulgaria. Diagn. Microbiol. Infect. Dis. **67:**319–326.
7. **Burette, A., and Y. Glupczynski.** 1995. *Helicobacter pylori*: the place of the new macrolides in the eradication of the bacteria in peptic ulcer disease. Infection **23**(Suppl. 1)**:**S44–S52.
8. **Cabrita, J., et al.** 2000. Features and trends in *Helicobacter pylori* antibiotic resistance in Lisbon area, Portugal (1990-1999). J. Antimicrob. Chemother. **46:**1029–1031.
9. **Cadranel, S., et al.** 1998. Detection of *Helicobacter pylori* infection in children with a standardized and simplified 13C-urea breath test. J. Pediatr. Gastroenterol. Nutr. **27:**275–280.
10. **Chey, W. D., and B. C. Wong.** 2007. American College of Gastroenterology guideline on the management of *Helicobacter pylori* infection. Am. J. Gastroenterol. **102:**1808–1825.
11. **Chisholm, S. A., E. L. Teare, K. Davies, and R. J. Owen.** 2007. Surveillance of primary antibiotic resistance of *Helicobacter pylori* at centres in England and Wales over a six-year period (2000-2005). Euro Surveill. **12**(7)**:**E3–E4.
12. **de Francesco, V., et al.** 2006. Primary clarithromycin resistance in Italy assessed on *Helicobacter pylori* DNA sequences by TaqMan real-time polymerase chain reaction. Aliment. Pharmacol. Ther. **23:**429–435.
13. **Dore, M. P., G. Leandro, G. Realdi, A. R. Sepulveda, and D. Y. Graham.** 2000. Effect of pretreatment antibiotic resistance to metronidazole and clarithromycin on outcome of *Helicobacter pylori* therapy: a meta-analytical approach. Dig. Dis. Sci. **45:**68–76.
14. **Duck, W. M., et al.** 2004. Antimicrobial resistance incidence and risk factors among *Helicobacter pylori*-infected persons, United States. Emerg. Infect. Dis. **10:**1088–1094.
15. **Dzierzanowska-Fangrat, K., et al.** 2001. Primary resistance to clarithromycin in clinical strains of *Helicobacter pylori* isolated from children in Poland. Int. J. Antimicrob. Agents **18:**387–390.
16. **Gatta, L., N. Vakil, G. Leandro, F. Di Mario, and D. Vaira.** 2009. Sequential therapy or triple therapy for *Helicobacter pylori* infection: systematic review and meta-analysis of randomized controlled trials in adults and children. Am. J. Gastroenterol. **104:**3069–3079.
17. **Gisbert, J. P., and J. M. Pajares.** 2005. *Helicobacter pylori* "rescue" therapy after failure of two eradication treatments. Helicobacter **10:**363–372.
18. **Glupczynski, Y.** 1996. Culture of *Helicobacter pylori* from gastric biopies and antimicrobial susceptibility testing, p. 17–32. *In* A. Lee and F. Megraud (ed.), *Helicobacter pylori*: techniques for clinical diagnosis and basis research. Saunders, London, United Kingdom.
19. **Glupczynski, Y., F. Megraud, M. Lopez-Brea, and L. P. Andersen.** 2001. European multicentre survey of in vitro antimicrobial resistance in *Helicobacter pylori*. Eur. J. Clin. Microbiol. Infect. Dis. **20:**820–823.
20. **Goossens, H., et al.** 2008. Achievements of the Belgian Antibiotic Policy Coordination Committee (BAPCOC). Euro Surveill. **13**(46)**:**pii=19036. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19036.
21. **Graham, D. Y., et al.** 1992. Factors influencing the eradication of *Helicobacter pylori* with triple therapy. Gastroenterology **102:**493–496.
22. **Janssen, M. J., et al.** 2006. *Helicobacter pylori* antibiotic resistance in a Dutch region: trends over time. Neth. J. Med. **64:**191–195.
23. **Kalach, N., et al.** 2007. *Helicobacter pylori* primary resistant strains over 11 years in French children. Diagn. Microbiol. Infect. Dis. **59:**217–222.
24. **Kato, S., et al.** 2002. Antibiotic resistance of *Helicobacter pylori* strains in Japanese children. J. Clin. Microbiol. **40:**649–653.
25. **Kobayashi, I., et al.** 2007. Changing antimicrobial susceptibility epidemiology of *Helicobacter pylori* strains in Japan between 2002 and 2005. J. Clin. Microbiol. **45:**4006–4010.
26. **Koletzko, S., et al.** 2006. Prospective multicentre study on antibiotic resistance of *Helicobacter pylori* strains obtained from children living in Europe. Gut **55:**1711–1716.
27. **Malfertheiner, P., et al.** 2007. Current concepts in the management of *Helicobacter pylori* infection: the Maastricht III consensus report. Gut **56:** 772–781.
28. **Marshall, B. J., and J. R. Warren.** 1984. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet **i:**1311-1315.
29. **McMahon, B. J., et al.** 2003. The relationship among previous antimicrobial use, antimicrobial resistance, and treatment outcomes for *Helicobacter pylori* infections. Ann. Intern. Med. **139:**463–469.
30. **Megraud, F.** 2001. Resistance of *Helicobacter pylori* to antibiotics and its impact on treatment options. Drug Resist. Updat. **4:**178–186.
31. **Megraud, F., et al.** 1999. Antimicrobial susceptibility testing of *Helicobacter pylori* in a large multicenter trial: the MACH 2 study. Antimicrob. Agents Chemother. **43:**2747–2752.
32. **Miendje Deyi, V. Y., C. Van Den Borre, and V. Fontaine.** 2010. Comparative evaluation of 3 selective media for primary isolation of *Helicobacter pylori*

from gastric biopsies under routine conditions. Diagn. Microbiol. Infect. Dis. **68:**474–476.
33. **Miendje Deyi, V. Y., et al.** 2011. Marching cohort of *Helicobacter pylori* infection over two decades (1988–2007): combined effects of secular trend and population migration. Epidemiol. Infect. **139:**572–580.
34. **National Committee for Clinical Laboratory Standards.** 2000. Performance standards for antimicrobial susceptibility testing and approved standard M7-A5. Informational supplement M100-S10. National Committee for Clinical Laboratory Standards, Wayne, PA.
35. **Ndip, R. N., et al.** 2008. *Helicobacter pylori* isolates recovered from gastric biopsies of patients with gastro-duodenal pathologies in Cameroon: current status of antibiogram. Trop. Med. Int. Health **13:**848–854.
36. **Nishizawa, T., H. Suzuki, and T. Hibi.** 2009. Quinolone-based third-line therapy for *Helicobacter pylori* eradication. J. Clin. Biochem. Nutr. **44:**119–124.
37. **Oleastro, M., et al.** 2003. Real-time PCR assay for rapid and accurate detection of point mutations conferring resistance to clarithromycin in *Helicobacter pylori*. J. Clin. Microbiol. **41:**397–402.
38. **Osato, M. S., R. Reddy, S. G. Reddy, R. L. Penland, and D. Y. Graham.** 2001. Comparison of the E-test and the NCCLS-approved agar dilution method to detect metronidazole and clarithromycin resistant *Helicobacter pylori*. Int. J. Antimicrob. Agents **17:**39–44.
39. **Oyedeji, K. S., S. I. Smith, A. O. Coker, and A. O. Arigbabu.** 2009. Antibiotic susceptibility patterns in *Helicobacter pylori* strains from patients with upper gastrointestinal pathology in western Nigeria. Br. J. Biomed. Sci. **66:**10–13.
40. **Parsons, H. K., M. J. Carter, D. S. Sanders, T. Winstanley, and A. J. Lobo.** 2001. *Helicobacter pylori* antimicrobial resistance in the United Kingdom: the effect of age, sex and socio-economic status. Aliment. Pharmacol. Ther. **15:**1473–1478.
41. **Peterson, W. L., et al.** 1993. Clarithromycin as monotherapy for eradication of *Helicobacter pylori*: a randomized, double-blind trial. Am. J. Gastroenterol. **88:**1860–1864.
42. **Prazeres, M. P., et al.** 2002. *Helicobacter pylori* primary resistance to metronidazole and clarithromycin in Brazil. Antimicrob. Agents Chemother. **46:** 2021–2023.
43. **Raymond, J., D. Lamarque, N. Kalach, S. Chaussade, and C. Burucoa.** 2010. High level of antimicrobial resistance in French *Helicobacter pylori* isolates. Helicobacter **15:**21–27.
44. **Storskrubb, T., et al.** 2006. Antimicrobial susceptibility of *Helicobacter pylori* strains in a random adult Swedish population. Helicobacter **11:**224–230.
45. **Teare, L., T. Peters, S. Saverymuttu, R. Owen, and I. Tiwari.** 1999. Antibiotic resistance in *Helicobacter pylori*. Lancet **353:**242.
46. **Tolia, V., W. Brown, M. El Baba, and C. H. Lin.** 2000. *Helicobacter pylori* culture and antimicrobial susceptibility from pediatric patients in Michigan. Pediatr. Infect. Dis. J. **19:**1167–1171.
47. **Vakil, N., and F. Megraud.** 2007. Eradication therapy for *Helicobacter pylori*. Gastroenterology **133:**985–1001.
48. **Van der Wouden, E. J., J. C. Thijs, A. A. Van Zwet, W. J. Sluiter, and J. H. Kleibeuker.** 1999. The influence of in vitro nitroimidazole resistance on the efficacy of nitroimidazole-containing anti-*Helicobacter pylori* regimens: a meta-analysis. Am. J. Gastroenterol. **94:**1751–1759.
49. **van Doorn, L. J., et al.** 2001. Accurate prediction of macrolide resistance in *Helicobacter pylori* by a PCR line probe assay for detection of mutations in the 23S rRNA gene: multicenter validation study. Antimicrob. Agents Chemother. **45:**1500–1504.
50. **Vecsei, A., et al.** 2010. Time trends of *Helicobacter pylori* resistance to antibiotics in children living in Vienna, Austria. Helicobacter **15:**214–220.
51. **Wang, G., and D. E. Taylor.** 1998. Site-specific mutations in the 23S rRNA gene of *Helicobacter pylori* confer two types of resistance to macrolide-lincosamide-streptogramin B antibiotics. Antimicrob. Agents Chemother. **42:**1952–1958.
52. **Wolle, K., A. Leodolter, P. Malfertheiner, and W. Konig.** 2002. Antibiotic susceptibility of *Helicobacter pylori* in Germany: stable primary resistance from 1995 to 2000. J. Med. Microbiol. **51:**705–709.
53. **Zullo, A., et al.** 2007. Primary antibiotic resistance in *Helicobacter pylori* strains isolated in northern and central Italy. Aliment. Pharmacol. Ther. **25:**1429–1434.